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The Mutagenesis of Benzo(a)pyrene on Yeast

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ABSTRACT

Benzo(a)pyrene (BaP) is one of many polycyclic aromatic hydrocarbons (PAHs), which are pollutant by-products of incomplete combustion that have been detected in the air, soils, sediments, water, and char-grilled or smoked foods. Recurrent exposure is a cause for concern, because a number of PAHs, such as BaP, have been identified as probable human carcinogens by the EPA. The purpose of this study was to test the mutagenic effects of BaP on a model organism: *Saccharomyces cerevisiae* (yeast). DNA was isolated from a 37 C, BaP-treated culture, amplified by the polymerase chain reaction (PCR), digested with the Hae III and Hind III restriction enzymes, and separated by electrophoresis on a 1.5% agarose gel. The DNA was then stained and the banding patterns were compared. The presence or absence of bands would indicate a mutation. Although it was expected that BaP would mutate the yeast DNA, no alteration was detected in the DNA sequence that was amplified.

Keywords: *Benzo(a)pyrene (BaP), polycyclic aromatic hydrocarbon (PAH), Saccharomyces cerevisiae, polymerase chain reaction (PCR), restriction enzyme, electrophoresis.*

INTRODUCTION

Benzo(a)pyrene (BaP) belongs to a group of compounds known as polycyclic aromatic hydrocarbons (PAHs), which are recalcitrant environmental pollutants composed of two or more fused aromatic rings. BaP is one of the more frequently studied PAHs due to its mutagenic and carcinogenic effects observed in the laboratory. The U.S. Environmental Protection Agency (EPA) has classified it as a Group B2 substance—probable human carcinogen, and it is number 9 on the 2003 Comprehensive Environmental Response Compensation, and Liability Act (CERCLA) priority list of hazardous substances (EPA, 1999; ATSDR, 2003).

Benzo(a)pyrene (see Fig. 1) is a solid compound with a pale yellow plate or needle-like crystal structure (ATSDR, 1995). It is produced as a by-product of incomplete combustion of organic matter, with the major source involving the burning of wood. Other sources of BaP, and PAHs in general, include volcanoes, coke oven emissions, tobacco and cigarette smoke, automobile exhausts, or any other process that employs the combustion of fossil fuels.

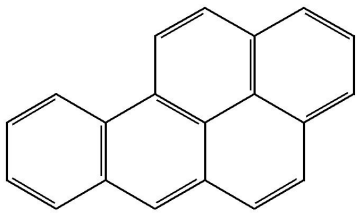

Figure 1. The molecular structure of benzo(a)pyrene.

Compounds have been detected in the air, water, food, sediments and soil (ATSDR, 1995). Therefore, human exposure is unavoidable and is an increasing cause for concern.

Health effects among humans are difficult to ascertain, because BaP and other PAHs do not occur singly, but in association with other compounds (ATSDR, 1995). However, studies have shown a higher incidence of lung cancer and related diseases among people with increased exposure to PAHs (ATSDR, 1995). Animal studies corroborate these findings, and propose further effects of recurrent exposure to PAHs. Development of gastrointestinal tract tumors, lung cancer, skin tumors, and reproductive problems are just a sample of the afflictions induced by specific testing of BaP and other PAHs on animals (IARC, 1983).

Benzo(a)pyrene and other PAHs have been found to be procarcinogens, meaning that they induce carcinogenic effects only after they have been metabolized (Simmon, 1979a). During metabolism, BaP may be oxidized and transformed into epoxide compounds (Stowers and Anderson, 1985). The electrophilic quality of the epoxides enables them to covalently bind to DNA, with a greater affinity for guanine (Braithwaite, Wu and Wang, 1998) than for other nucleotides. If the metabolite-DNA adducts interfere with other cellular processes, they could then initiate carcinogenesis.

One model organism that can be effective for mutagenic analysis, and is extensively used for studies in genetics and with environmental chemicals, is yeast (*Saccharomyces cerevisiae*). Yeasts are eukaryotes and possess internal

structures, such as chromosomes and a differentiated nucleus containing a nucleolus, like cells of higher organisms (Simmon, 1979b). They are also inexpensive, readily available, and allow for a sizeable number to be studied. Analyzing a larger population would permit the observation of rarer changes, especially when looking for mutagenic effects of a chemical such as BaP. For these reasons, yeast is a valuable tool for research and can lead to further studies, which could then have potential human interest.

The objective of this study is to examine the effects of a specific environmental chemical, BaP, on the yeast genome. Because it has displayed mutagenic and/or carcinogenic effects in past studies, it is expected that BaP will induce mutations in the yeast DNA, especially at guanine sites. To test this hypothesis, a yeast culture will be exposed to BaP and allowed to metabolize and reproduce. Its DNA will be extracted and copied by the Polymerase Chain Reaction (PCR), which is a technique chosen on account of its sensitivity in amplifying extremely small amounts of DNA. The copied DNA will be fragmented by the use of a restriction enzyme and separated by agarose gel electrophoresis to provide an observable arrangement of the DNA. Mutations will present themselves with the presence and/or absence of bands when comparing the BaP-treated and control samples.

MATERIALS AND METHODS

Saccharomyces cerevisiae wild type HAO strain was purchased from Carolina Biological Supply (Burlington, NC) and cultured in two test tubes, each containing 10 ml of nutrient broth. One test tube was treated with 3.0 µg/l of BaP (Fisher Scientific) and incubated at 37 C for forty-eight hours. Due to the low water solubility of BaP, dimethyl sulfoxide (DMSO) was used as a solvent in a concentration of 0.0003%. To attain this concentration, 1.0 mg of BaP was dissolved in 1.0 ml of DMSO and 0.3 ml of that solution was pipetted into 1.0 L of H_2O. A 0.1 ml quantity was then added to the 10 ml yeast culture. The second test tube served as a control, and was also incubated for forty-eight hours at 37 C.

Yeast DNA was then extracted from both cultures using the Epicentre® MasterPure™ Yeast DNA Purification Kit. According to its protocol (Epicentre®, 2004), yeast cells were harvested from a saturated 1.5 ml sample and lysed by the Yeast Cell Lysis Solution. DNA was then precipitated with isopropanol, isolated and suspended in TE buffer (10mM Tris-HCl [pH 8.0], 1 mM EDTA). The yeast DNA-buffer solution was stored at 4 C.

Amplification of the DNA was achieved with the Polymerase Chain Reaction (PCR). The reagents needed for this process were acquired from the Epicentre® FailSafe™ PCR System. As suggested by its protocol (Epicentre®, 2004), PCR reactions (50 µl) contained 4 µl of DNA template, 1 µM of each primer, 18 µl of sterile water, 1 µl of FailSafe™ PCR Enzyme Mix, and 25 µl of FailSafe™ PCR 2X PreMix A. The primers selected, which code for the RNA guanine 7-methyltransferase gene (ABD1), were synthesized at Integrated DNA Technologies (Coralville, IA) with the following sequences: 5'-CGG GAT CCA GGA GGA AAG TAG AAT GTC AAC CAA ACC A-3' and 5'-GGG GTA CCT CAG TTG GGC TTT ACG CTT TC-3'. The RNA gene exists in all eukaryotes and plays an important role in mRNA metabolism.

Thirty cycles of amplification were performed under the following conditions: 45 seconds denaturation at 95 C; 45 seconds annealing at 62 C; 20 minutes extension at 70 C.

Amplified DNA was then loaded with the Hae III and Hind III restriction enzymes (New England BioLabs, Beverly, MA; Carolina Biological Supply, Burlington, NC) onto a prepared 1.5% agarose gel for electrophoresis. The Hae III restriction enzyme recognizes and "cuts" the GG^CC sequence in DNA. Although this recognition sequence is not present in the primers themselves, it does occur twice throughout the complete ABD1 gene. The Hind III restriction enzyme recognizes and "cuts" the A^AGCTT sequence in DNA. It is also not present in the primer sequence, but occurs once in the ABD1 gene.

Electrophoresis was run at 80 volts for 105 minutes. The separated DNA bands were then made visible with Carolina Blu™ Stain. The DNA banding pattern of the BaP-treated and control samples were compared for indications of mutations.

RESULTS

Upon the completion of the PCR, a spectrophotometer was utilized to quantify the copied DNA. Absorbances indicated 0.220 µg/µl of yeast-control DNA (untreated yeast), 0.250 µg/µl of DMSO-control DNA (yeast in 0.0003% DMSO), and 0.230 µg/µl of experimental DNA (BaP-treated yeast in 0.0003% DMSO). These figures were used to determine the restriction enzyme units required for digestion.

Following electrophoresis (see Fig. 2), both the cut and uncut DNA produced similar banding patterns. In comparison to the molecular weight marker (lambda DNA) the bands were approximately 3,400 bp in length. This indicates that the Hae III restriction enzyme did not cut the DNA as it was intended to.

Because the Hae III restriction enzyme failed to cut, even though multiple attempts were made to produce the suitable conditions it requires, a second restriction enzyme, Hind III, was used. It worked successfully and produced two distinct bands that were approximately 2,000 and 1,500 bp in length (see Fig. 3).

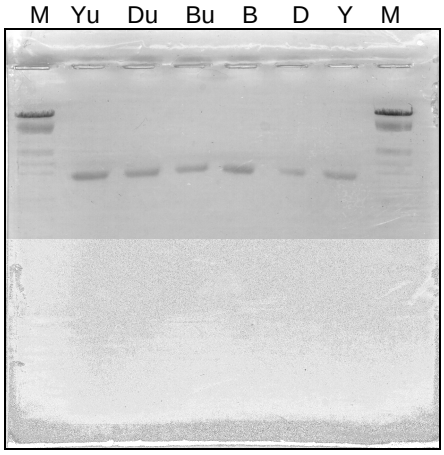

Figure 2. Electrophoresis was run on a 1.5% agarose gel with 2.5 µg each of lambda DNA (M) cut with EcoR I and Hind III, yeast-control DNA (Yu), DMSO-control DNA (Du), BaP-treated DNA (Bu), each uncut, and yeast-control DNA (Y), DMSO-control DNA (D), and BaP-treated DNA (B), each cut with Hae III.

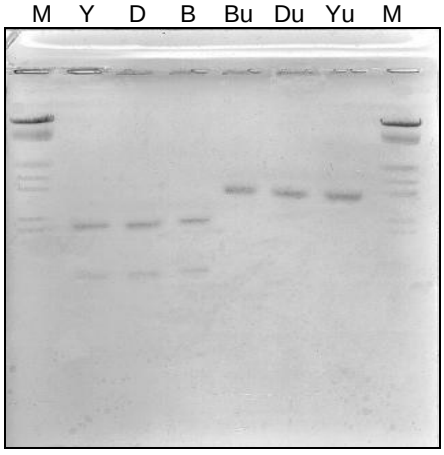

Figure 3. Electrophoresis was run on a 1.5% agarose gel with 2.5 µg each of lambda DNA (M) cut with EcoR I and Hind III, yeast-control DNA (Y), DMSO-control DNA (D), BaP-treated DNA (B), each cut with Hind III, and yeast-control DNA (Yu), DMSO-control DNA (Du), and BaP-treated DNA (Bu), each left uncut.

DISCUSSION

It was expected that a mutation would occur in the yeast genome in response to being treated with BaP. However, for the guanine 7-methyltransferase gene selected for this study, no alterations in its DNA sequence were detected. Possible reasons for this negative result include: a lack in DNA visibility, mutation occurrence in another part of the yeast genome, or simply, no mutation.

The Hae III restriction enzyme should have recognized and cut the unaltered gene at two places in the gene sequence. This would have formed three small bands that were 393, 342, and 126 bp in length, once sorted by gel electrophoresis. Unfortunately, it is very difficult to visualize such small fragments on the gel. They could have been present and altered in some way, but just not seen.

An alternative explanation would be that a mutation did occur, but in a different part of the yeast genome. Only the guanine 7-methyltransferase gene was amplified during the PCR process, so a modification in another part of the yeast DNA would not have been adequately available for detection. However, the size of the gene that was copied should have been 861 bp, but 3,400 bp were observed. This may have occurred if the PCR did not stop at the designated position, but continued to copy the DNA sequence.

The simplest reason for not observing a mutation would be that no mutation occurred in the yeast, as indicated by the banding pattern produced by the Hind III restriction enzyme. Several variables could have prevented this. One possibility could have been that there was no BaP uptake by yeast cells, or if there was, it may have caused those cells to die. If this were the case, then DNA would have only been isolated from surviving cells. In addition, BaP present in viable cells may not have been effective in binding to the DNA.

The next step in this research would be to investigate the negative result that was obtained. If, at some point, a mutation is detected, it should be sequenced to determine the type of mutation that developed. The process would then need to be repeated to ensure that it was not an inadvertent modification. The significant changes that take place in the yeast DNA could then potentially indicate similar alterations in human DNA, because studies have suggested that yeast genes can provide clues to human gene function (Chervitz et al., 1998)

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